THE BIOLOGICAL ACTIVITY OF 26-HYDROXY-DERIVATIVES OF CHOLECALCIFEROL IN VITAMIN D-DEFICIENT RATS

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1. Introduction

In the course of our work investigating the stereochemistry and biological activity of the metabolite of vitamin D_3, 25,26-dihydroxycholecalciferol 25,26$(OH)_2D_3$, isolated from porcine [1] and human [2] plasma, we have synthesized [3] the C-25 epimers of the latter and established [4] their absolute configuration. One of these epimers, 25R,26- or 25S,26$(OH)_2D_3$, must be identical with the natural product.

The biological activity of 25R,26- and 25S,26$(OH)_2D_3$ was examined [3] in rachitic rats and the latter epimer was found superior to the former in elevating serum phosphorus and in the cure of rickets. These results led us to undertake the synthesis [5] of the unknown epimeric 26-hydroxy-derivatives of vitamin D_3, (25R)- and (25S)-26-OH-D_3, which may be helpful in defining more clearly the relation between the 26-hydroxyl and biological activity.

This paper reports the biological activity of (25R)- and (25S)-26-OH-D_3 on bone and gut in vitamin D-deficient rats. We describe also the effects of 25R,26- and 25S,26$(OH)_2D_3$ on intestinal calcium transport, not reported in [3]. These results make comparisons between the 25R and 25S epimers of either 26-OH-D_3 or 25,26$(OH)_2D_3$ possible.

2. Materials and methods

Biological methods were detailed in [6].

2.1. *Chemical compounds*

2.1.1. 25R,26- and 25S,26$(OH)_2D_3$

These compounds were prepared as in [3,4].

2.1.2. (25R)- and (25S)-26-OH-D_3

These compounds were obtained by chemical synthesis from (25R)- and (25S)-26-hydroxycholesterol [5,7].

2.2. *Animals and diets*

Weanling Sprague-Dawley male rats were fed for 4 weeks on a synthetic vitamin D-free diet containing 0.47% Ca^{2+} and 0.30% P. Then the animals were kept for a further week either on a low Ca^{2+} (< 0.02%) vitamin D-free diet for experiments measuring intestinal Ca^{2+} transport and bone Ca^{2+} mobilization, or a low phosphorus (< 0.02%) vitamin D-free diet for experiments investigating bone calcification and serum phosphorus variation.

2.3. *Analytical procedures*

Calcium was measured by atomic absorption (Perkin-Elmer 303) in presence of 1% of $LaCl_2$. P_i was determined according to [8]. Radioactivity was measured using a liquid scintillation counter (Intertechnique SL 40) and $^{45}Ca^{2+}$ was counted in Instagel solution (Packard).

2.4. *In vitro intestinal Ca^{2+} transport*

This was measured by the everted gut sac technique [9] and the results expressed as the $^{45}Ca^{2+}$ concentration inside/outside ratio.

2.5. *Bone Ca^{2+} mobilization*

The increase of serum Ca^{2+} 24 h after injection is regarded as resulting from the mobilization of bone Ca^{2+}.

2.6. *Calcification of bone*

This was measured by the line-test [10] 1 week after the administration of compounds.

2.7. *Serum phosphorus*

This was determined 1 week after the administration of compounds.

2.8. *Administration of compounds*

Epimers of either 26-OH-D_3 or 25,26$(OH)_2D_3$ (250 ng) were dissolved in ethanol (50 μl) and injected intravenously. Controls were tested with ethanol (50 μl).

2.9. *Statistical analysis*

Analysis for statistical significance was performed by Wilcoxon's nonparametric test.

3. Results

Table 1 shows the effects of 250 ng (corresponding to 10 IU) of (25R)-26-OH-D_3 and (25S)-26-OH-D_3 on bone. In vitamin D- and phosphorus-deficient rats only the 25R epimer is effective in elevating serum phosphorus and in stimulating bone calcification, while in vitamin D- and Ca^{2+}-deprived animals neither 25R nor 25S induce bone resorption.

Tables 2 and 3 illustrate the effects of 250 ng C-25 epimers of either 26-OH-D_3 or 25,26$(OH)_2D_3$ on intestinal Ca^{2+} transport in vitamin D- and Ca^{2+}-deficient rats. While 25R,26- and 25S,26$(OH)_2D_3$ are both

Table 1
Effects of the epimers of 26-OH-D_3 on bone Ca^{2+} mobilization, serum phosphorus and bone calcification

Compound	Bone Ca^{2+} mobilization (serum calcium mg/l) Mean ± SD	Serum phosphorus (mg/l) Mean ± SD	Bone calcification (Epiphyseal calcification score) Mean ± SD
25-OH-D_3 (125 ng)	75.75 ± 4.79 (4) $W_4^4 = 10$ $p = 0.05$	38.57 ± 2.99 (7) $W_7^7 = 28$ $p < 0.01$	3.12 ± 0.05 (8) $W_8^7 = 28$ $p < 0.01$
(25R)-26-OH-D_3 (250 ng)	48.00 ± 3.40 (6) $W_6^4 = 14$ NS	32.00 ± 3.00 (7) $W_7^7 = 31$ $p < 0.01$	1.87 ± 0.95 (8) $W_8^7 = 29$ $p < 0.01$
(25S)-26-OH-D_3 (250 ng)	50.33 ± 2.87 (6) $W_6^4 = 21$ NS	26.14 ± 2.79 (7) $W_7^7 = 47.5$ NS	0 ± 0 (8)
Control (50 μl ethanol)	47.23 ± 2.22 (4)	26.86 ± 2.48 (7)	0.07 ± 0.19 (7)

Table 2
Effects of the epimers of 26-OH-D_3 on intestinal Ca^{2+} transport

Control (50 μl ethanol)	Inside/outside ratio. Mean ± SD		
	25-OH-D_3 (250 ng)	(25R)-26-OH-D_3 (250 ng)	(25S)-26-OH-D_3 (250 ng)
0.87 ± 0.05 (4)	1.93 ± 0.08 (4) $W_4^4 = 10$ $p = 0.05$	1.03 ± 0.03 (6) $W_6^4 = 10$ $p = 0.01$	0.95 ± 0.07 (6) $W_6^4 = 15$ NS

Table 3
Effects of the epimers of $25,26(OH)_2D_3$ on intestinal Ca^{2+} transport

Control (50 μl ethanol)	Inside/outside ratio. Mean ± SD		
	25-OH-D_3 (250 ng)	25R,26$(OH)_2D_3$ (250 ng)	25S,26$(OH)_2D_3$ (250 ng)
0.9 ± 0.01 (4)	1.90 ± 0.11 (4) $W_4^4 = 10$ $p = 0.05$	0.99 ± 0.05[a] (6) $W_6^4 = 11.5$ $p < 0.05$	1.15 ± 0.14[a] (6) $W_6^4 = 10$ $p = 0.01$

[a] Difference between 25R,26- and 25S,26$(OH)_2D_3$ was significant ($p = 0.05$)

effective (the latter significantly ($p = 0.05$) more than the former) only (25R)-26-OH-D_3 stimulates Ca^{2+} absorption.

4. Discussion

Our results demonstrate that among the 26-hydroxy-derivatives of vitamin D_3 the biological activity depends upon the configuration at C-25 and the 25R epimer: (25R)-26-OH-D_3 is more effective on bone and gut than the 25S one: (25S)26-OH-D_3. We have investigated [3] the relation between C-25 configuration and biological response with respect to the 25,26-dihydroxy-derivatives of vitamin D_3: 25R,26- and 25S,26$(OH)_2D_3$ and found the latter biologically superior to the former.

The fact that the more effective epimers of either 26-OH-D_3 or $25,26(OH)_2D_3$: (25R)-26-OH-D_3 and 25S,26$(OH)_2D_3$, respectively, bear opposite signs of conventional configuration at C-25, can be probably explained by their metabolic relationship and the rules of nomenclature [11].

Indeed, 26-hydroxy-derivatives of vitamin D_3 are very likely to be converted by a hepatic hydroxylase system [12] into 25,26-dihydroxy-derivatives (similar metabolic hydroxylation at C-25 has been reported for 24-hydroxyvitamin D_3 [13]) and this reaction generally proceeds with retention [14] of spatial configuration. But introduction of the hydroxyl into the asymmetric centre C-25 induces inversion of conventional signs (R or S) in accordance with the rules of nomenclature [11].

From the foregoing it may be concluded that metabolic hydroxylation of (25R)- and (25S)-26-OH-D_3 should yield 25S,26- and 25R,26$(OH)_2D_3$, respectively. In other words, (25R)-26-OH-D_3 and 25S,26$(OH)_2D_3$ on the one hand, (25S)-26-OH-D_3 and 25R,26$(OH)_2D_3$ on the other hand, should have similar spatial configuration and activity.

Our biological results support this conclusion.

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